Exercise intervention during pregnancy induces promoter methylation alterations in cord blood

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Research

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Abstract

Background: Environmental events and nutritional conditions may induce permanent DNA methylation changes *in utero* during the sensitive period and these adaptive changes may be 'memorized' and have a lasting impact on adult disease later in life. The potential plasticity of DNA methylation also enables reprogramming, depending on exposure to nutritional, chemical, and environmental factors. We determined the effects of exercise training during pregnancy on epigenetic modifications in offspring.

Results: Twenty-four subjects were chosen from a previous clinical study. Overweight/obese pregnant women (body mass index <28/≥28kg/m^2) who had an uncomplicated pregnancy at <12^{+6} weeks of gestation were randomly allocated to either an exercise or a control group. Patients allocated to the exercise group performed 3 exercise bouts per week (at least 30 min/session with a rating of perceived exertion between 12-14) via a cycling program that was initiated within 3 days of randomization until 37 weeks of gestation. Patients allocated to the control group continued their usual daily activities. Maternal blood, as well as umbilical cord blood samples, were collected and DNA methylation levels were determined by Illumina MethylationEPIC microarray.

Nine CpG sites and corresponding genes *UMAD1*, *RPA3*, *PLAGL2*, *POFUT1*, *SPATA17*, *GPATCH2*, *CEP170*, *MPHOSPH10*, *MCEE*, as well as *MRGPRD* showed differential trends in control and exercise groups in maternal blood. Furthermore, four specific genes in umbilical cord blood were differentially methylated in the exercise group compared to the sedentary controls ($p < 0.01$).

Conclusions: DNA methylation in maternal blood is dynamically altered during pregnancy and remodeled by exercise training. Thus, intrauterine environmental exposures play an important role in fetal programming. Epigenetic mechanisms involving changes in DNA methylation may provide an explanation for the phenomenon. Our findings shed light on understanding of long-term effects of *in utero* exposures on the epigenetic landscape of the offspring.

Background

Obesity characterized with excess accumulation of white adipose tissue increases the prevalence of gestational diabetes mellitus (GDM). Exposure to intrauterine hyperglycemia increases the long-term sequelae including cardiovascular abnormalities and metabolic syndrome in adult offspring [1–3]. As early as two decades ago, Dr. David Barker provided evidence that nutritional exposures in utero has a lasting impact on offspring's health status in adulthood [4]. This emphasizes the critical role of early intrauterine environmental exposures and highlights the potential origins of adult metabolic diseases.

Conventional strategies for the management of obesity during pregnancy such as diet modifications are often insufficient. Exercise has long been considered to improve insulin sensitivity and as an adjuvant therapy in the management of Type 2 diabetes in non-pregnant subjects. Type 2 diabetes and GDM share the similarity of insulin resistance and thus exercise therapy may also applied to the GDM population. The International Federation of Gynecology and Obstetrics (FIGO) recommends appropriate, personally

adapted, physical activity for all women with gestational diabetes mellitus. Our previous study provided evidence that exposure to exercise intervention during pregnancy improves pregnancy outcomes in overweight and obese pregnant women [5].

The underlying mechanism by which environmental influences affect the offspring is still obscure. Epigenetic modifications have been proposed to be a potential mechanism, thereby contributing to our understanding of long-term influences of in utero exposure [6–8]. DNA methylation is one of the major epigenetic modifications. CpG dinucleotide (5-methyl cytosine followed by guanosine) is the dominant type of methylation in mammals and CpG dinucleotides are usually clustered (also known as CpG islands) in the promoter regions of genes, which thereby regulate gene expression by interfering transcription factors access to DNA template.

DNA methylation is involved in imprinting, mammalian development, and genomic stability maintenance. A number of cell type-specific DNA methylation patterns are believed to be established in the embryonic stage and the potential plasticity of DNA methylation enables reprogramming, depending on exposures to environmental, nutritional and chemical factors. Intrauterine exposures may induce adaptive DNA methylation changes, through which the in utero exposures may have a lasting impact on future disease manifestation in adult life. We here illustrate the role of epigenetic modifications in umbilical cord blood of offspring in response to intrauterine exposures induced by exercise training.

Research Design And Methods

Study Participants and sample collection

The study was approved by the Institutional Review Board of Peking University First Hospital. All studies were performed according to the declaration of Helsinki. Informed consent was obtained from all participants. We conducted a randomized clinical trial at Peking University First Hospital from December 2014 through July 2016. The study protocol was reported previously [5]. Briefly, the study recruited nonsmoking women age > 18 years with a singleton pregnancy who met the criteria for overweight/obese status (body mass index < 28/≥28 kg/m^2) and had an uncomplicated pregnancy at < 12^{+6} weeks of gestation. The women were randomly allocated to either exercise or a control group. Gestational age was assessed based on the last menstrual period and early ultrasound. Patients did not have contraindications to physical activity. Patients allocated to the exercise group performed exercise 3 times per week (at least 30 min/session with a rating of perceived exertion between 12–14) via a cycling program that was initiated within 3 days of randomization until 37 weeks of gestation. All the exercise sessions occurred at Peking University First Hospital under supervision. Patients assigned to the control group continued their usual daily activities. Both groups received standard prenatal care, albeit without special dietary recommendations.

Blood was drawn from the antecubital vein after the participant had fasted for at least 8 hours, but not > 14 hours. Blood was collected in sterile vacutainer tubes preloaded with heparin. There was at least 24

hours between the last exercise bout and the time of blood draw. Blood was centrifuged at 1800 g at 4 °C for 10 minutes within 4 hours of collection; subsequently, the plasma was separated and glucose was measured immediately. The remaining plasma was stored at -80 °C for later analysis of insulin and lipid parameters (triglyceride, total cholesterol, and low- and high-density lipoprotein cholesterol). Insulin was measured using radioimmunoassay commercial kits (Beifang Institute of Biochemical Technology, Beijing, China). The insulin resistance index was calculated according to the homeostasis model of assessment: fasting plasma insulin (μU/mL) × fasting glucose (mmol/L)/22.5 [9]. Fasting lipid profiles were measured using an automatic analyzer (7600; Hitachi High-Technologies Corp, Tokyo, Japan) in the Department of Clinical Laboratory, Peking University First Hospital. Biochemical tests were repeated at 25 and 36 gestational weeks. Umbilical cord blood samples were obtained and immediately stored once the baby was delivered. Twelve participants from each group were recruited in the following DNA methylation array analysis. Clinical characteristics of participants and pregnancy outcomes were recorded and presented in Table 1.

DNA methylation sample preparation

DNA was extracted from 2 ml of whole blood from each sample using Blood DNA Midi Kit (Omega). The total amount of DNA was determined by spectrophotometry. The DNA samples were sent to the Beijing KPS Biotechnology Co., Ltd and stored at -20 °C. Quality check was performed by Nanodrop Spectrophotometer and resolution on a 0.8% agarose gel. Bisulfite convert the genomic DNA samples using the Zymo EZ DNA Methylation Kit (Zymo Research).

Illumina MethylationEPIC microarray

The EPIC platform has probes targeting 866,836 cytosine positions on the human genome, of which 863,904 (99.7%) are CpG dinucleotides and 2932 (0.3%) CNG targets. Additionally, there are 59 probes targeting SNP sites to allow sample matching and 636 probes for sample-dependent and sample-independent quality control. EPIC probes are principally located at promoters (54%), followed by gene bodies (30%) and then intergenic regions (16%), and 19% and 18% of all EPIC probes are located in CpG islands and CpG island shores, respectively.

According to Illumina's specification, DNA was hybridized to Illumina MethylationEPIC microarrays. Briefly, the bisulfite-converted DNA samples were denatured, neutralized and prepared for amplification after overnight incubation. Amplified DNA samples were enzymatically fragmented and precipitated. Subsequently, precipitated DNA samples were resuspended and dispensed onto BeadChips. BeadChips were incubated in the Illumina Hybridization Oven to hybridize the samples onto the BeadChips. The BeadChips were stained and scanned following the instructions. Data analysis was performed by R bioconductor package minfi [10] for methylation intensity calculation and limma [11] for differential analysis.

Data processing

For methylation analysis, IDAT files were loaded into the R (3.4.3) environment using the Bioconductor minfi package (1.26.2). Quality control was performed and CpG sites with detection p value < 0.05 were kept. Signals were then normalized using quantile method [12]. The level of DNA methylation at a particular CpG site (also called the methylation beta-value (β)) was calculated by taking the ratio of the methylated intensity (M) to unmethylated intensity (U) signal, using the formula: $\beta = M/(M + U)$. A β-value of 0 represents a completely unmethylated CpG site and a β-value approaching 1 represents a fully methylated CpG site.

Probes on sex chromosomes were removed, as well as those identified as cross-hybridizing with other genomic locations or SNP-associated [13]. A set of 683,126 probes was obtained and used for exploratory and differential methylation analysis.

Differential methylation analysis

In order to assess differences in methylation between groups, the original β values were converted to M-values via the logit transformation. Differentially methylated probes were detected using the R bioconductor limma (3.36.3) package. Probes with adjusted p value < 0.05 were considered to be differentially methylated. The Benjamini–Hochberg method was used to adjust the p values and ensure that the false discovery rate was < 0.05. The corresponding gene list was derived from the gene annotations associated with the probes.

Statistics

Clinical results were statistically analyzed by SPSS 20.0. All values are presented as mean ± SD. Two-tailed unpaired Student's t-test were performed. Regarding differential methylation analysis, probes with adjusted p value < 0.05 were considered to be differentially methylated. The Benjamini–Hochberg method was used to adjust the p values and ensure that the false discovery rate was < 0.05.

Results

Twelve participants from the exercise group and the control group were recruited for the DNA methylation array analysis (Fig. 1). There were no significant differences of age, weight, height, prepregnancy body mass index, gestational age and fasting plasma glucose at study entry between exercise and control groups (Table 1). The exercise intervention increased moderate exercise levels at 25 and 36 gestational weeks ($p < 0.05$). No vigorous exercise was reported in participants. There was no difference in walking amount and total exercise load between two groups (Table 2). The exercise intervention group gained less weight from study entry to 25^{+6} weeks of gestation compared to the control group ($p < 0.05$). No difference of weight gain in the later stage of gestation (26^{+1} weeks to 36^{+6} weeks) was reported in the current study. The participants in exercise and control groups had the equal homeostatic model assessment for insulin resistance (HOMA-IR), triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) profiles (Supplementary Table).

Dynamic alterations of DNA methylation profiles during pregnancy in maternal blood

In order to illustrate the DNA methylation alterations in different trimesters in maternal blood, we categorized DNA methylation changes in 1st, 2nd and 3rd trimesters to four groups according to the trend. We defined DNA methylation baseline level of 1st trimester as “0”, DNA methylation level increase between trimesters as “1” and DNA methylation level decrease between trimesters as “-1”. Thus, we detected four patterns of DNA methylation profiles (0. 1. -1; 0. 1. 1; 0. -1. 1; 0. -1. -1) in either control or exercise participants (Fig. 2A, Fig. 2B). Red lines indicate significant changes of differentially methylated positions (DMPs) ($p < 0.05$) and grey lines indicate non-significant changes (Fig. 2A, Fig. 2B).

There were 26573 DMPs in the control group and 15913 DMPs in the exercise group for 0. 1. -1 trend (Fig. 2C). DMPs were defined as both a significant change for 2nd vs. 1st trimester ($p < 0.05$) and also a significant change for 3rd vs. 2nd trimester ($p < 0.05$). Among them, 2562 DMPs in control group and 1345 DMPs in exercise group were significantly altered in 0. 1. -1 pattern (Fig. 2C). Similarly, for 0. -1. 1 trend, we found 35363 DMPs (2014 significant DMPs) in the control group and 14635 DMPs (1076 significant DMPs) in the exercise group respectively (Fig. 2C). Interestingly, there were fewer DMPs for 0. 1. 1 and 0. -1. -1 trends both in control and exercise groups (Fig. 2A, Fig. 2B). Only two significant DMPs (cg13769674, cg08530065) in the control and one significant DMP (cg04260608) in the exercise-trained group for 0. 1. 1 trend were identified. Similarly, one DMP (cg08586855) and two DMPs (cg12394567, cg02033213) were found for 0. -1. -1 trend in control and exercise groups, respectively (Fig. 2C).

Differentially methylated regions (DMRs) are genomic regions with different DNA methylation status across the biological samples and regarded as possible functional regions involved in gene transcriptional regulation. Compared to 1st trimester in maternal blood, 17781 DMRs were revealed in 2nd trimester in the control group, among which 1293 DMRs were located in promoters. 6565 DMRs were identified in the exercise group and 615 DMRs were located in promoters. 4530 DMRs and 3145 DMRs were found in 3rd trimester compared to 2nd trimester in control and exercise groups, respectively. 477 DMRs and 280 DMRs were located in promoters accordingly (Fig. 3A).

DNA methylation levels of specific genes were altered by exercise in maternal blood

We next examined the DMPs with opposite trends in control and exercise groups in order to determine whether exercise training affects the DNA methylation trends in maternal blood. We compared the significant DMPs between control and exercise groups with four opposite trend combinations (trends 0. -1. 1 and 0. 1. -1; trends 0. 1. -1 and 0. -1. 1; trends 0. 1. 1 and 0. -1. -1; trends 0. -1. -1 and 0. 1. 1, respectively). There were no significant CpG sites identified in the latter two opposite trend combinations (trends 0. 1. 1 and 0. -1. -1; trends 0. -1. -1 and 0. 1. 1). Three CpG sites and corresponding genes UMAD1 (UBAP1-MVB12-associated (UMA) domain containing 1, cg12309238), RPA3 (replication protein A3, cg12309238), PLAGL2 (PLAG1 like zinc finger 2, cg25811820) and POFUT1 (protein O-fucosyltransferase 1, cg25811820) showed significantly differential trends in control (0. -1. 1) and exercise (0. 1. -1) groups in maternal blood (Fig. 4). Six CpG sites and corresponding genes including SPATA17 (spermatogenesis associated 17, cg17026642), GPATCH2 (G-patch domain containing 2, cg17026642), CEP170

(centrosomal protein 170, cg08258520), MPHOSPH10 (M-phase phosphoprotein 10, cg20854010), MCEE (methylmalonyl-CoA epimerase, cg20854010) as well as MRGPRD (MAS related GPR family member D, cg11903239) demonstrated opposite trends 0. 1. -1 (control) and 0. -1. 1 (exercise) during pregnancy (Fig. 4).

The global DNA methylation profile of cord blood is different from that of maternal blood

Next, we compared the DNA methylation profiles in maternal blood and cord blood. We studied the epigenome-wide variation in DNA methylation with principal component analysis (PCA), which shows that the leading axis of epigenomic variation reflects the tissue type (Fig. 5A). Dots represent blood samples and are colored accordingly. The heatmaps show DNA methylation comparisons between cord blood and maternal blood in different trimesters (Fig. 5B, Fig. 5C, Fig. 5D). The differential genes of maternal and cord blood were then grouped into three categories using Gene Ontology (GO) system: biological process (describing the larger cellular or physiological role carried out by the gene, coordinated with other genes) and cellular component (describing the location in the cell where the gene product executes its function) and molecular function (describing the molecular activity of a gene) (Fig. 5E). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway demonstrated that “metabolic pathways” was the most significant pathway enrichment in differential genes comparing maternal and cord blood (Fig. 5F). The corresponding proteins of differential genes of maternal and cord blood were analyzed using protein-protein interactions (PPIs) network, whereby the physical contacts of high specificity is established between potential protein molecules (Fig. 5G).

Compared to 3rd trimester in maternal blood, 82676 DMRs were demonstrated in cord blood in control, among which 9464 DMRs were located in promoters. 78318 DMRs were identified in the exercise group and 9130 DMRs were located in promoters (Fig. 3B).

Maternal exercise alters gene-specific DNA methylation levels in cord blood

The methylation microarray data showed that five specific CpG sites cg02878244, cg02819231, cg02505749, cg11660360, cg03084276 were significantly differentially methylated in the exercise group compared the control group in cord blood (Fig. 6A). These CpG sites were located within the CpG islands of Transcription Start Site (TSS) of four specific genes including developing brain homeobox 1 (DBX1), F-box and leucine rich repeat protein 2 (FBXL2), potassium two pore domain channel subfamily K member 9 (KCNK9) and prostaglandin reductase 1 (PTGR1) (Fig. 6C).

KEGG pathway analysis revealed that “aldosterone synthesis and secretion” was the most significant pathway enrichment in cord blood from the exercise versus the control groups (Fig. 6B).

Discussion

The general pattern of DNA methylation in human fetal germ cells during development has been comprehensively analyzed [14]. Nevertheless, the patterns of DNA methylation during pregnancy, as well

as DNA methylation of the neonate, have not been established. DNA methylation is thought to be mitotically stable, with environmental events and nutritional conditions after birth unlikely to alter DNA methylation changes in adult tissues [15]. Nevertheless, our study provides evidence that DNA methylation could be dynamically altered during pregnancy, as well as in response to exercise training, in maternal blood. Interestingly, there were few significant CpG sites showing increasing or decreasing trends during the whole pregnancy. The majority CpG sites have two patterns of DNA methylation waves, either firstly increasing from the first to second trimester and then decreasing from the second to third trimester, or vice versa. Additionally, more differential DMRs were found in the early half of the pregnancy.

Previous study demonstrated that acute gene activation induced by a bout of exercise is associated with a rapid change in DNA methylation in another adult somatic tissue, skeletal muscle [16]. Specifically, exercise triggered marked hypomethylation on respective promoter of peroxisome proliferator-activated receptor gamma, coactivator 1 a (PGC1α), pyruvate dehydrogenase kinase, isoenzyme 4 (PDK4) and peroxisome proliferator-activated receptor δ (PPAR-δ), genes playing important roles in cell metabolism [16]. In the current study, we reported dynamic changes in maternal blood in response to exercise training. In contrast to skeletal muscle biopsies, blood samples are relatively easy to obtain from pregnant woman. Thus we are unable to determine whether exercise training elicited effect on DNA methylation in skeletal muscle of pregnant women. In a previous study, DNA methylation alterations were demonstrated immediately after an acute bout of exercise, revealing an exercise intensity-dependent response. The current study showed DNA methylation changes based on a moderate physical activity level over a relatively longer period.

Exercise induced significant DNA methylation alterations of specific CpG sites and corresponding genes including UMAD1 (cg12309238), RPA3 (cg12309238), PLAGL2 (cg25811820) and POFUT1 (cg25811820), SPATA17 (cg17026642), GPATCH2 (cg17026642), CEP170 (cg08258520), MPHOSPH10 (cg20854010), MCEE (cg20854010) as well as MRGPRD (cg11903239). Among these genes, MRGPRD was reported to play an important role in protecting osteocytes and preventing bone loss through preventing mitochondrial breakdown in osteocytes [17]. In the current study, we demonstrated dynamic, opposing alterations of DNA methylation levels of MRGPRD in maternal blood induced by exercise. This provided evidence that exercise might trigger functional changes in critical factors by regulating gene expression through DNA methylation mechanism.

Our study provided evidence that metabolic pathways markedly differ between maternal blood and umbilical cord blood. This finding raises an issue related to tissue specificity when using cord blood. Umbilical cord blood, like adult blood, is a heterogeneous tissue (e.g. monocytes neutrophils, eosinophils, basophils, B cells, NK cells, and T cells). The proportions of cells with nuclei fluctuate greatly throughout fetal development and may vary significantly depending on the gestational age [18]. DNA methylation levels are affected by cell type proportion fluctuation across gestational age. It has been shown blood cell type methylation markers were segregated into two clusters by gestational age. The two clusters of DNA methylation measurement were characterized by proportions of granulocytes, lymphocytes, and B cells in

cord blood, which were determined by gestational age [19]. Therefore, the cell type proportion differences partially contribute to the DNA methylation variations in maternal blood and cord blood.

Our previous study reported differentially methylated genes in neonates exposed to the intrauterine hyperglycemia using the array-based method [20]. Altered DNA methylation levels of Rho guanine nucleotide exchange factor 11 (ARHGEF11) were negatively correlated with glucose levels and neonatal birth weight, which emphasized the potential influence of intrauterine environment and maternal nutrition status on DNA methylation. The current study further illustrated the DNA methylation alterations could be induced by maternal nutritional and metabolic status triggered by exercise. KEGG pathway showed that “aldosterone synthesis and secretion” was the most significant pathway enrichment in cord blood comparing exercise and control groups. Aldosterone is a steroid hormone synthesized in and secreted from the outer layer of the adrenal cortex. Aldosterone plays an important role in the regulation of systemic blood pressure through the absorption of sodium and water. Our study sheds light on the potential metabolic disease pathogenesis in offspring, which might be related to the maternal life style during pregnancy.

Previous studies demonstrated that caffeine mimics exercise-induced expression of genes related to mitochondrial function in L6 myotubes [21]. Moreover, a time-course study of caffeine exposure of L6 myotubes suggests that exercise mimetics may trigger promoter hypomethylation of specific genes [16]. Nevertheless, the mechanism by which maternal exercise alters the intrauterine environment and concomitantly influences the fetal DNA methylation establishment remains unknown.

Notably, the glucose and lipid profiles were unaltered between the exercise intervention and control groups in the current study. Nevertheless, the exercise intervention decreased weight gain in the first half of gestation. The importance of intensity, duration and frequency of exercise in a critical window of pregnancy needs to be emphasized, which may affect DNA methylation marks of offspring. This question warrants further studies since intensity, duration and frequency are important components of exercise prescription as well as weight management through exercise in gestational diabetes mellitus management.

The effectiveness and modality of physical activity during pregnancy in prevention of gestational diabetes mellitus, as well as the underlying mechanism is still obscure. DNA methylation appears to sense environmental changes, regulates gene expression, and prepares the body to respond to changes in the milieu. Environmental factors have a dynamic effect on the level of DNA methylation in the promoter regions of key genes. Our data provide a comprehensive picture of the dynamic alterations of DNA methylation during pregnancy in maternal blood, as well as the effect of exercise on fetal DNA methylation profiles. Changes in DNA methylation may be an early event in reprogramming the metabolic profile in human somatic tissues, as well as cord blood. Our results provides evidence for implementing exercise intervention strategies for disease management in order to modify epigenetic marks.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Peking University First Hospital (reference numbers: 2014[726], 2019[45]). All studies were performed according to the declaration of Helsinki. Informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

JY and HY designed research; CW and YW conducted the exercise intervention; JY and CW processed samples; JY and HY wrote the paper; HY had primary responsibility for final content. HY is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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References

1. Dabelea D, Hanson RL, Bennett PH, Roumain J, Knowler WC, Pettitt DJ: **Increasing prevalence of Type II diabetes in American Indian children**. *Diabetologia* 1998, **41**(8):904-910.

2. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD *et al*: **Hyperglycemia and adverse pregnancy outcomes**. *N Engl J Med* 2008, **358**(19):1991-2002.
3. Simeoni U, Barker DJ: **Offspring of diabetic pregnancy: long-term outcomes**. *Semin Fetal Neonatal Med* 2009, **14**(2):119-124.
4. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME: **Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease**. *BMJ* 1989, **298**(6673):564-567.
5. Wang C, Wei Y, Zhang X, Zhang Y, Xu Q, Sun Y, Su S, Zhang L, Liu C, Feng Y *et al*: **A randomized clinical trial of exercise during pregnancy to prevent gestational diabetes mellitus and improve pregnancy outcome in overweight and obese pregnant women**. *American journal of obstetrics and gynecology* 2017, **216**(4):340-351.
6. Thompson RF, Fazzari MJ, Niu H, Barzilai N, Simmons RA, Greally JM: **Experimental intrauterine growth restriction induces alterations in DNA methylation and gene expression in pancreatic islets of rats**. *J Biol Chem* 2010, **285**(20):15111-15118.
7. Simmons RA: **Developmental origins of adult disease**. *Pediatr Clin North Am* 2009, **56**(3):449-466, Table of Contents.
8. Breton CV, Byun HM, Wenten M, Pan F, Yang A, Gilliland FD: **Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation**. *Am J Respir Crit Care Med* 2009, **180**(5):462-467.
9. Muniyappa R, Lee S, Chen H, Quon MJ: **Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage**. *American journal of physiology Endocrinology and metabolism* 2008, **294**(1):E15-26.
10. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, Irizarry RA: **Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays**. *Bioinformatics* 2014, **30**(10):1363-1369.
11. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK: **limma powers differential expression analyses for RNA-sequencing and microarray studies**. *Nucleic acids research* 2015, **43**(7):e47.
12. Touleimat N, Tost J: **Complete pipeline for Infinium((R)) Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation**. *Epigenomics* 2012, **4**(3):325-341.
13. Pidsley R, Zotenko E, Peters TJ, Lawrence MG, Risbridger GP, Molloy P, Van Djik S, Muhlhausler B, Stirzaker C, Clark SJ: **Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling**. *Genome biology* 2016, **17**(1):208.
14. Guo H, Hu B, Yan L, Yong J, Wu Y, Gao Y, Guo F, Hou Y, Fan X, Dong J *et al*: **DNA methylation and chromatin accessibility profiling of mouse and human fetal germ cells**. *Cell research* 2017, **27**(2):165-183.
15. Reik W, Dean W, Walter J: **Epigenetic reprogramming in mammalian development**. *Science* 2001, **293**(5532):1089-1093.

16. Barres R, Yan J, Egan B, Treebak JT, Rasmussen M, Fritz T, Caidahl K, Krook A, O'Gorman DJ, Zierath JR: **Acute exercise remodels promoter methylation in human skeletal muscle**. *Cell metabolism* 2012, **15**(3):405-411.
17. Kitase Y, Vallejo JA, Gutheil W, Vemula H, Jahn K, Yi J, Zhou J, Brotto M, Bonewald LF: **beta-aminoisobutyric Acid, l-BAIBA, Is a Muscle-Derived Osteocyte Survival Factor**. *Cell reports* 2018, **22**(6):1531-1544.
18. Ygberg S, Nilsson A: **The developing immune system - from foetus to toddler**. *Acta Paediatr* 2012, **101**(2):120-127.
19. Braid SM, Okrah K, Shetty A, Corrada Bravo H: **DNA Methylation Patterns in Cord Blood of Neonates Across Gestational Age: Association With Cell-Type Proportions**. *Nursing research* 2017, **66**(2):115-122.
20. Yan J, Su R, Zhang W, Wei Y, Wang C, Lin L, Feng H, Yang H: **Epigenetic alteration of Rho guanine nucleotide exchange Factor 11 (ARHGEF11) in cord blood samples in macrosomia exposed to intrauterine hyperglycemia**. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet* 2019:1-10.
21. Ojuka EO, Jones TE, Han DH, Chen M, Holloszy JO: **Raising Ca2+ in L6 myotubes mimics effects of exercise on mitochondrial biogenesis in muscle**. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2003, **17**(6):675-681.

Tables

Table 1. Baseline characteristics of participants

Characteristics	Exercise group n=12	Control group n=12	p
Age, y	32.92 ± 1.93	31.92 ± 4.40	0.48
Weight, kg	70.47 ± 7.56	70.46 ± 10.62	1.00
Height, cm	162.92 ± 5.21	162.17 ± 7.53	0.78
p-BMI, kg/m^2	26.61 ± 3.22	26.68 ± 2.35	0.95
Gestational age, wk	9 ± 2	10 ± 2	0.46
Fasting plasma glucose at study entry, mmol/L	4.88 ± 0.41	4.86 ± 0.48	0.91

Data presented as mean ± SD; p-BMI, prepregnancy body mass index.

Table 2. Physical activity levels in overweight and obese pregnant women randomized to exercise intervention or control groups

	Exercise group n=12	Control group n=12	p
Vigorous exercise, metabolic equivalents of task min/wk			
At study entry	0	0	
At 25 wk gestation	0	0	
At 36 wk gestation	0	0	
Moderate exercise, metabolic equivalents of task min/wk			
At study entry	15 ± 52	3 ± 9	0.42
At 25 wk gestation	151 ± 103	8 ± 22	< 0.0005
At 36 wk gestation	92 ± 49	9 ± 23	< 0.005
Walking, metabolic equivalents of task min/wk			
At study entry	617 ± 616	494 ± 485	0.59
At 25 wk gestation	435 ± 202	664 ± 627	0.26
At 36 wk gestation	465 ± 397	381 ± 331	0.68
Total, metabolic equivalents of task min/wk			
At study entry	632 ± 613	496 ± 485	0.55
At 25 wk gestation	586 ± 273	672 ± 632	0.68
At 36 wk gestation	557 ± 427	390 ± 323	0.43

Figures

Figure 1

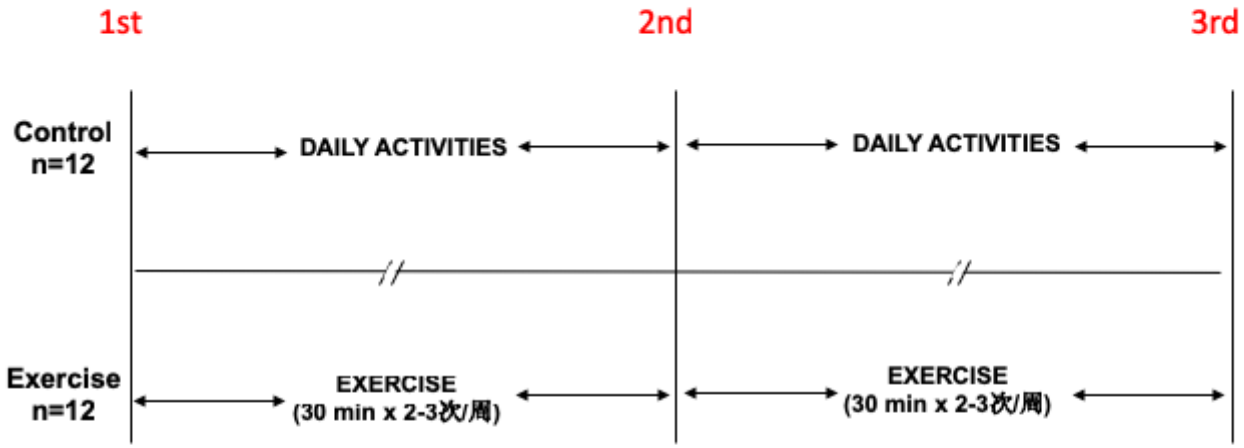

Figure 1

Exercise intervention illustration. The study recruited nonsmoking women age >18 years with a singleton pregnancy who met the criteria for overweight/obese status (body mass index <28/≥28kg/m2) and had an uncomplicated pregnancy at <12+6 weeks of gestation were randomly allocated to either exercise or a control group. Patients did not have contraindications to physical activity. Patients allocated to the exercise group were assigned to exercise 3 times per week (at least 30 min/session with a rating of perceived exertion between 12-14) via a cycling program begun within 3 days of randomization until 37 weeks of gestation. Those in the control group continued their usual daily activities.

Figure 2

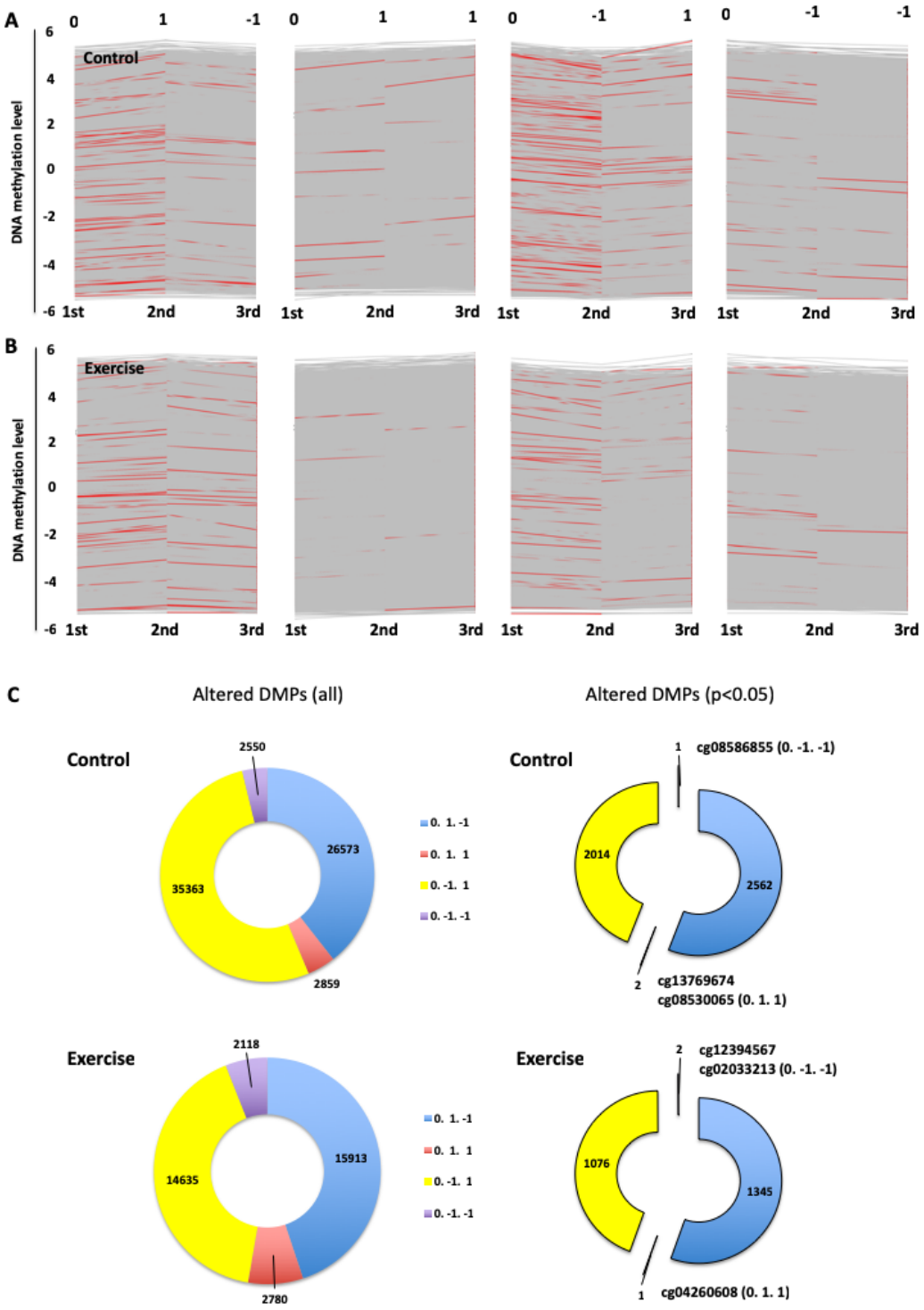

Figure 2

Dynamic alterations of DNA methylation profiles in different trimesters. (A) (B) In order to illustrate the DNA methylation alterations in different trimesters, we categorized DNA methylation changes during the pregnancy to four groups according the trend. We defined DNA methylation baseline level of 1st trimester as “0”, DNA methylation level increase between trimesters as “1” and DNA methylation level decrease between trimesters as “-1”. There will be four trends of DNA methylation profiles (0, 1, -1; 0, 1, 1; 0, -1, 1; 0,

-1, -1) in either control or exercise participants. Red lines stand for significant changes of differentially methylated positions (DMPs) ($p<0.05$) and grey lines stand for non-significant changes. (C) The numbers of altered DMPs in control group and exercise group were shown. The significant DMPs in four individual trends were defined as significant change for 2nd vs. 1st trimester ($p<0.05$) and also significant change for 3rd vs. 2nd trimester ($p<0.05$). Among them, there were 2562, 2, 2014, 1 significant DMPs in control group for 0. 1. -1, 0. 1. 1, 0. -1. 1 and 0. -1. -1 trends, respectively. Similarly, there were 1345, 1, 1076, 2 significant DMPs in exercise group for 0. 1. -1, 0. 1. 1, 0. -1. 1 and 0. -1. -1 trends.

Figure 3

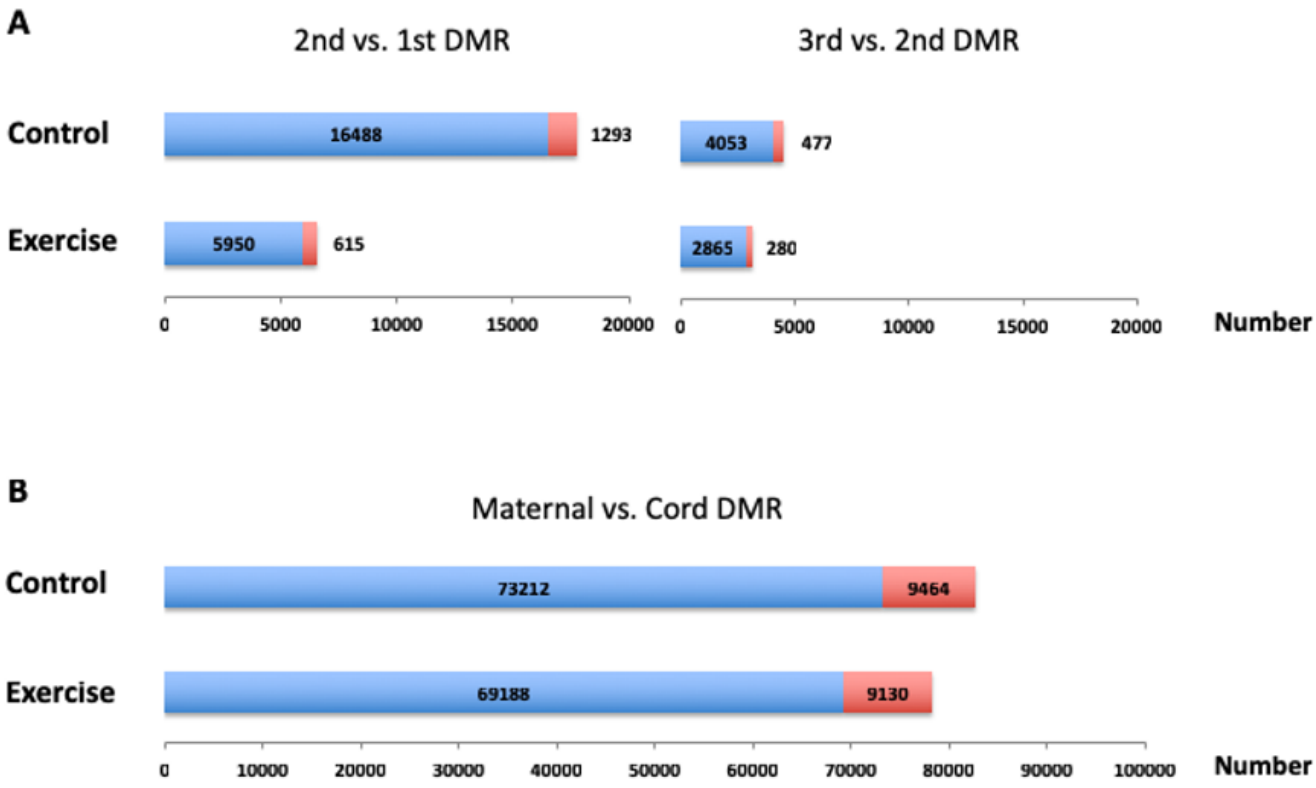

Figure 3

Dynamic alterations of DNA methylation profiles between different trimesters in maternal blood and between maternal and cord blood. (A) The numbers of DMRs were demonstrated. Compared to 1st trimester in maternal blood, total 17781 DMRs were revealed in 2nd trimester in control, among which 1293 DMRs were located in promoters (red bar), 16488 in non-promoters (blue bar). 6565 DMRs were identified in the exercise group and 615 DMRs were located in promoters (red bar), 5950 in non-promoters (blue bar). 4530 DMRs and 3145 DMRs were found in 3rd trimester compared to 2nd trimester in control and exercise groups, respectively. 477 DMRs and 280 DMRs were located in promoters accordingly (red

bar). (B) The numbers of DMRs were demonstrated. Compared to 3rd trimester in maternal blood, 82676 DMRs were demonstrated in cord blood in control, among which 9464 DMRs were located in promoters (red bar). 78318 DMRs were identified in the exercise group and 9130 DMRs were located in promoters (red bar).

Figure 4

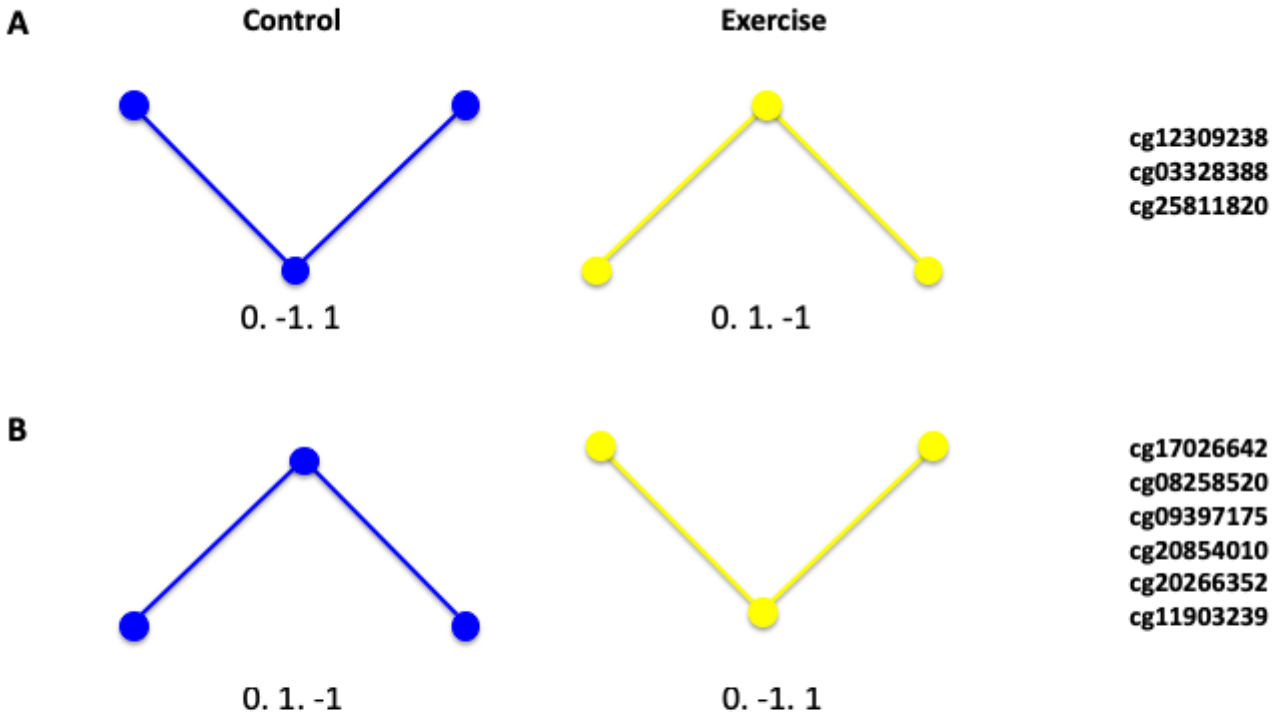

Figure 4

DNA methylation levels of specific genes were altered by exercise in maternal blood. We defined DNA methylation baseline level of 1st trimester as “0”, DNA methylation level increase between trimesters as “1” and DNA methylation level decrease between trimesters as “-1”. (A) We examined the DMPs in control and exercise groups with opposite trends of 0. -1. 1 and 0. 1. -1. Three CpG sites were shown significantly differential trends in control (0. -1. 1) and exercise (0. 1. -1) groups in maternal blood. (B) Six CpG sites were demonstrated in opposite trends 0. 1. -1 and 0. -1. 1 during pregnancy.

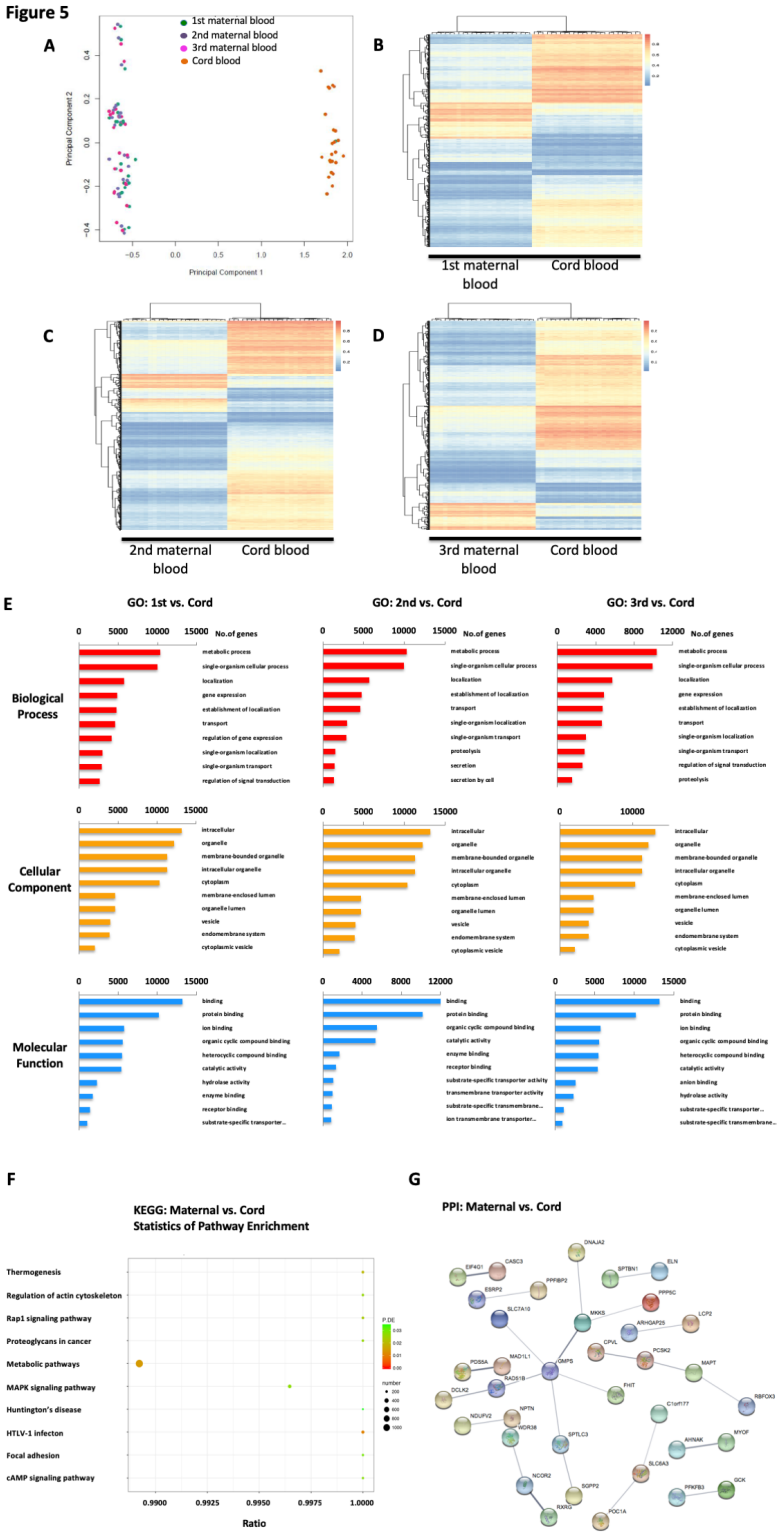

Figure 5

The global DNA methylation profile of cord blood is different from that of maternal blood. (A) The epigenome-wide variation in DNA methylation with principal component analysis (PCA) shows that the leading axis of epigenomic variation reflects the tissue type. Dots represent blood samples and are colored accordingly. (B)(C)(D) The heatmaps showed DNA methylation comparisons between cord blood and maternal blood in different trimesters. (E) The differential genes of maternal and cord blood were

then grouped into three categories using Gene Ontology (GO) system: biological process (describing the larger cellular or physiological role carried out by the gene, coordinated with other genes) and cellular component (describing the location in the cell where the gene product executes its function) and molecular function (describing the molecular activity of a gene). (F) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway demonstrated that “metabolic pathways” was the most significant pathway enrichment in differential genes comparing maternal and cord blood. (G) The corresponding proteins of differential genes of maternal and cord blood were analyzed using protein-protein interactions (PPIs) network, by which the physical contacts of high specificity established between potential protein molecules.

Figure 6

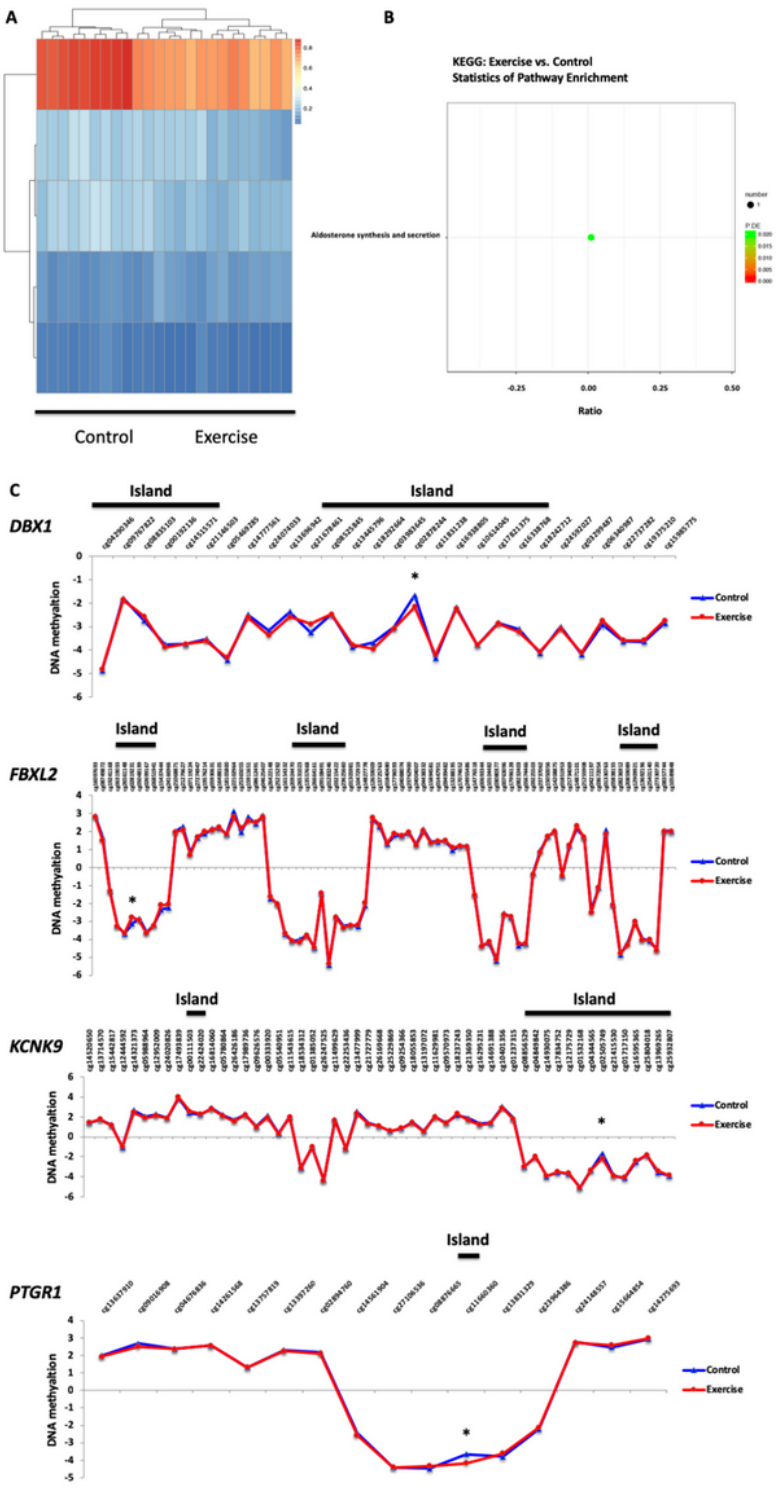

Figure 6

Maternal exercise alters gene-specific DNA methylation levels in cord blood. (A) The heatmap showed DNA methylation comparisons between control and exercise groups in cord blood. (B) KEGG pathway demonstrated that “aldosterone synthesis and secretion” was the significant pathway enrichment comparing exercise and control groups in cord blood. (C) DNA methylation levels of specific genes. Four specific genes developing brain homeobox 1 (DBX1), F-box and leucine rich repeat protein 2 (FBXL2),

potassium two pore domain channel subfamily K member 9 (KCNK9) and prostaglandin reductase 1 (PTGR1). The control group was represented in blue and exercise group was in red. It only showed the relative positions of CpG sites and specific CpG sites within islands. * indicated significant levels of $p<0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- OnlineSupplementalMaterials.docx